THE RESISTANCE OF THE FRESHWATER SNAIL, *PHYSA HETEROSTROPHA* (SAY) TO SEA WATER.

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The freshwater snail *Physa heterostropha* has been observed in the brackish water of Barnegat Bay near the mouth of the Metedeconk River, Bay Head, N. J. An interesting problem presented itself; how far into brackish water can these freshwater snails migrate? To test this some preliminary experiments were attempted in the summer of 1928. By gradually increasing the salinity of the water *Lymnæa stagnalis appressa* and *L. palustris* (from Michigan) were made to live in 25 per cent. sea water. *Physa heterostropha* (from Philadelphia) died soon after being placed in 10 per cent. sea water. This stock was probably weak. These experiments were summed up in a preliminary report (Richards, 1929).

The summer experiments were of a very preliminary nature and rather crude; so additional and more accurate experiments were begun in the fall of the same year. The work, which was concentrated upon the one species, *P. heterostropha*, seemed to divide itself into two parts: first the experiments dealing with the ability of the species as a whole to withstand gradual and sudden increases in the concentration of sea water; and second, the reactions of the various stocks of the same species to the salt water.

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Methods and Procedure.

In order to determine whether there were any racial differences between various lots of the same species, several sets of controls were kept.

Control.	Locality.	Date collected.
A	Schuylkill River, Fairmount Park, Philadelphia, Pa.	Oct. 16, 1928
B	Schuylkill River, Fairmount Park, Philadelphia, Pa.	Oct. 27, 1928
C	Branch of Cobbs Creek, Haverford, Pa.	Oct, 28, 1928
D	Davies Lake, Cape May Point, N. J.	Nov. 11, 1928
E	Davies Lake, Cape May Point, N. J.	Mar. 30, 1929
F	Wissahickon Creek, Fort Washington, Pa.	Apr. 6, 1929
G	Pond, Botanical Gardens, University of Pennsylvania, Philadelphia, Pa.	Aug. 7, 1929
H	Davies Lake, Cape May Point, N. J.	Aug. 11, 1929

The snails were brought to the Zoölogical Laboratory of the University of Pennsylvania, where they were placed in culture jars. The water used in these experiments was tap water which had been allowed to stand for several days in order to eliminate some of the chlorine so abundant in Philadelphia tap water. In all cases the snails were allowed to live in the fresh water for one week in order to adjust themselves to any possible change. The mortality of the control was found to be highest during the first week.

After the first week some snails were placed in 5 per cent sea water,[1] and a 5 per cent. increase per week was continued until the water reached 30 per cent.; at this point serious effects of the salts were noticed in the behavior of the snails and therefore some were allowed to remain in this concentration with the hope that they might become adjusted to it; others were placed in stronger sea water.

Additional experiments were attempted in which the 5 per cent. increase was made at intervals of two days and one day. Other snails were placed directly in 5 per cent., 10 per cent., 15 per cent., 20 per cent. and 25 per cent. sea water in order to see if they could stand the sudden change.

The sea water used to make up these solutions in all the experiments except part 3 was taken from the vivarium of the University of Pennsylvania. This water had been brought from Ocean City, N. J., some months previous. In the experiments of part 3, which were conducted at Cape May Point, N. J., water from the ocean was used.

The specific gravity of the vivarium water is kept relatively constant at 1.023 (at 17.5°C.). The principal difference between

[1] Normal sea water being taken as 100 per cent.

vivarium water and normal sea water was found to be in the hydrogen ion concentration. Normal sea water (off New Jersey) has a pH between 8.1 and 8.3, whereas the water in the tank is more acid, varying from pH 7.8 to 7.9. The acidity is probably due to the acid excrement and dead organic matter from the marine animals in the aquarium.

The snails were fed green lettuce several times a week, and the old lettuce and excrement removed at frequent intervals, the procedure recommended by Crabb (1929) for the best growth of pond snails.

The temperature was not regulated; it usually lay within the interval between 15° and 20° C.

Results.

Part 1, *5 per cent. Increase in Concentration Every Week.*

Physa lived actively in water as strong as 25 per cent. sea water. Above 25 per cent. the harmful effects of the salts were noted. Above this concentration the activity of the snails was considerably decreased. Snails from control *D* (Cape May Point, N. J.) were considerably more active in 30 per cent. sea water than those from any other control.

Snails were allowed to remain in water of between 25 per cent. and 30 per cent. for several weeks in order to see if they gradually became adjusted to this concentration. However no such adjustment seemed to take place during the several weeks, although throughout the experiments the snails from control *D* were more active in the brackish water than any of the others.

After various intervals the snails were placed in higher concentrations (up to 50 per cent.). In all cases they died, except those in *D*, which were inactive but still alive. Upon being transferred to 5 per cent. sea water they soon revived and were as active as ever.

These experiments are summarized in the following two tables. Table I. deals with the experiments with the 5 per cent. increase in concentration as far as 30 per cent., at which point this method was discontinued.

TABLE I.

SHOWING RESULTS OF EXPERIMENTS IN WHICH THE 5 PER CENT. INCREASE IN CONCENTRATION WAS MADE EVERY WEEK.

The number of snails alive at the beginning and end of each week is given. The summation of all experiments is also given.[1]

		5%	10%	15%	20%	25%	30%
A control	33–19	13–11	11–11	11–11	11–11	11–11	11–11
Experiment		5–5	5–5	5–5	5–5	5–5	5–5
B control	70–67	52–52	52–47	47–45	45–45	40–40	40–40
Experiment		10–10	10–10	10–10	10–10	10–10	10–10
C control	149–139	100–95	95–93	83–83	83–83	83–83	83–83
Experiment		15–15	15–15	15–15	15–15	15–15	15–15
D control	50–50	50–50	50–50	50–50	50–50	50–50	50–50
Experiment		10–10	10–10	10–9	9–9	9–9	9–9
Summation controls	302–275	215–208	208–201	191–189	189–189	184–184	184–184
Experiments		40–40	40–40	40–39	39–39	39–39	39–39
% surviving controls	91.0%	92.3%	96.6%	98.9%	100%	100%	100%
Experiments		100%	100%	97.5%	100%	100%	100%

Table II. surveys the experiments in water of higher concentration than 30 per cent. Since the procedure following the period of attempted adjustment differed in the various experiments, and would be difficult to represent in strictly tabular form, the procedure and results are summarized in a few words in Table II.

Part 2, *5 Per Cent. Increase in Concentration at Intervals of Two Days.*

When the concentration was increased 5 per cent. every two days instead of every week, the results were practically the same. In these experiments also the snails from Davies Lake (*D* and *E*) showed a greater resistance to sea water than the other snails.

The results of these experiments are shown briefly in Table III.

[1] For the sake of comparison the following table is given showing the average specific gravity (at 17.5° C.) and average salinity (parts per thousand) of the various concentrations of sea water used throughout these experiments. The data were calculated from Knudsen's Hydrographical Tables.

Per Cent. Normal Sea Water.	Sp. Gr. (17.5° C.).	Salinity.	Per Cent. Normal Sea Water.	Sp. Gr. (17.5° C.).	Salinity.
5%	1.0007	1	30%	1.0085	12
10%	1.0020	2 to 3	35%	1.0105	14.5
15%	1.0040	4 to 5	50%	1.0130	17
20%	1.0050	6	100%	1.0230	30
25%	1.0070	8			

Table II.

Showing Experiments in Water of Higher Concentration than 30 Per Cent.

The number of snails alive at the end of the week in 30% is given, then the number of weeks that the snails were left in water of approximately 25 or 30%; the further experiments are then summarized in a few words.

	Number Alive in 30% (from Table 1).	Number of Weeks in 25–30%.	Further Experiments.
A control.....	11		11–11
Experiment 1...	5	1	Placed in 40%; death at end of one week.
B control.....	40		40–38
Experiment 1...	5	4	Placed in 35%; one dead at end of first week; others died during second week.
Experiment 2...	5	1	Increased to 35% in which three died; increased to 50%; all died.
C control.....	83		83–83
Experiment 1...	5	2	Concentration fluctuated considerably between 1.007 and 1.012; died in four weeks.
Experiment 2...	10	2	Active in 30%; alive but inactive in 35%; died in 50%.
D control.....	50		50–50
Experiments....	9	3	Active in 30% and 35%; placed in 50% alive but inactive at end of 24 hours; revived when placed in 5% sea water.

Table III.

Showing Results of Experiments with Increase of 5 Per Cent. in Salinity at Intervals of Two Days.

The number of snails alive at the beginning and end of each interval is shown

	5%	10%	15%	20%	25%	30%	35%	40%	50%
B Control...	8–8	8–8	8–8	8–8	8–8	8–8	8–8	8–8	8–8
Experiment..	5–5	5–5	5–5	5–5	5–5	5–5 fairly active	5–5 inactive	5–2 inactive	2–0 dead
C Control...	8–8	8–8	8–8	8–8	8–8	8–8	8–8	8–8	8–8
Experiment..	5–5	5–5	5–5	5–5	5–5	5–5 fairly active	5–5 inactive		5–0 dead
D Control...	21–21	21–21	21–21	21–21	21–21	21–21	18–18	18–18	18–18
Experiment..	10–10	10–10	10–10	10–10	10–9	9–9 active	9–9	9–9 fairly active	9–8 slightly active. Revived in fresh water.
E Control...	16–16	16–16	16–16	16–16	16–16	16–16	16–16	16–16	16–16
Experiment..	5–5	5–5	5–5	5–5	5–5	5–5	5–5	5–5 active	5–5 fairly active. Revived in fresh water.

Part 3, *5 Per Cent. Increase in Concentration at Intervals of One Day.*

There was no difference in the behavior of the snails when the increase was made at intervals of one day instead of two days. Those snails (*H*) from Davies Lake showed slightly greater resistance to sea water than those (*G*) from the pond in Philadelphia.

Part 4, *Sudden Change to Brackish Water.*

Physa was able to live after sudden transfers to 5 per cent., 10 per cent., 15 per cent., 20 per cent. and 25 per cent. sea water, but the Davies Lake snails (*D*, *E* and *H*) were the only ones to show signs of activity in 20 per cent. and 25 per cent. sea water.

Discussion.

Whether the concentration was increased 5 per cent. at intervals of one or two days or even a week did not seem to have any significant bearing on the ability of the snails to become acclimated to the sea water. Various workers have achieved complete acclimatization of freshwater organisms to sea water over a long period of time. Beaudant (1816) successfully acclimatized *Physa fontinalis* to sea water by very gradually increasing the salinity of the water over a period of more than six months. A very long period of time, and a very gradual increase in the concentration of the salt are probably necessary for the complete acclimatization of most freshwater organisms.

Hydrogen Ion Concentration.

The possibility that death might have been caused by changes in pH, rather than by increase in salinity, was considered. Walton and Wright (1926) have shown that *Lymnæa truncatula* can stand a variation in H-ion concentration from pH 6.0 to pH 8.6, and *L. peregra* from pH 5.8 to pH 8.8. All the solutions used in the experiments lie well within this range as the following table will show:

	pH
Tap water	7.2–7.4
Aquarium water	7.2–7.4
Control after one week	7.2–7.3
Sea water from Vivarium	7.8–7.9
Sea water (Cape May Point, N. J.)	8.1–8.3
25 per cent. sea water (diluted with aquarium water)	7.5–7.7

The pH was determined colorimetrically by the use of the indicators Cresol Red and Brom Thymol Blue, and was verified in a few cases electrolytically. *Physa* was able to live normally when transferred to the acid Cedar Swamp water from the Mullica River near Batsto, N. J. (pH 5.9); likewise the snails could live in synthetic alkaline water (pH 8.1).

These data serve to show that probably some effect of the salts present in sea water rather than the change in H-ion concentration causes the death of the snails.

A More Hardy Stock from Davies Lake.

Numerous indications throughout the experiments showed that the snails from Davies Lake, Cape May Point, N. J. (controls *D*, *E* and *H*) have more resistance than the snails from the other localities. They were more active than the others in 25–30 per cent. sea water; they were active in water as concentrated as 1.012 (at 17.5 °C.), showing a resistance not shared by the other stocks; they were the only stock to survive 50 per cent. sea water; here they were inactive, but soon revived when placed in 5 per cent. sea water; they were the only stock to show any signs of activity after a sudden transfer from fresh water to 20 per cent. and 25 per cent. sea water.

This evidence seems to show fairly well that this stock is more resistant to sea water than those taken from the vicinity of Philadelphia. Although several tests of water from Davies Lake showed a specific gravity of 1.000 at 4° C., during storms the waters of the lake are probably mixed with the salt water of Delaware Bay only 50 yards distant. The resistance of the Davies Lake stock may have been acquired during several generations.

Bailey (1929) has recorded *P. heterostropha* together with other freshwater snails in the brackish water of Chesapeake Bay, and as mentioned above, the writer has found the same species in Barnegat Bay, N. J.

Summary.

1. By gradually increasing the salinity, *Physa heterostropha* was made to live quite actively in 25 per cent. sea water.

2. *Physa* can live but is not active in concentrations between 30 per cent. and 40 per cent.

3. In water stronger than 40 per cent. all the snails died with the exception of the Davies Lake lot, which had retreated within their shells, but which revived when placed in 5 per cent. sea water.

4. Whether the 5 per cent. increase in concentration was made at intervals of one or two days, or even a week, seemed to make no difference in the ability of the snails to become acclimated to the sea water.

5. All the races survived a sudden change to 5 per cent., 10 per cent., 15 per cent., 20 per cent. and 25 per cent., but the Davies Lake stock was the only one active in 20 per cent. and 25 per cent., at the end of one week.

6. The snails from Davies Lake may be regarded as a stock which is more hardy to sea water than the other stocks used in the experiments.

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